



101/GB2004/004646

GB04/04636

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

REC'D 29 NOV 2004

WIPO PCT

**PRIORITY
DOCUMENT**
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated

Stephen Hordley

15 November 2004

BEST AVAILABLE COPY

Patents Act 1977
(Rule 16)**Request for grant of a patent**

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

**1/77**The Patent Office
Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference

95.82307/001

2. Patent application number
(The Patent Office will fill in this part)

0412544.9

4 JUN 2004

3. Full name, address and postcode of the
or of each applicant (underline all surnames)Camurus AB
Ideon, Gamma 1
Sölvegatan 41
SE-223 70 Lund
Sweden

Patents ADP number (if you know it)

8217762602

If the applicant is a corporate body, give
country/state of its incorporation

Sweden

4. Title of the invention

Compositions

5. Name of your agent (if you have one)

Frank B. Dehn & Co.

"Address for service" in the United Kingdom
to which all correspondence should be sent
(including the postcode)179 Queen Victoria Street
London
EC4V 4EL

Patents ADP number (if you know it)

166001 /

6. Priority: Complete this section if you are
declaring priority from one or more earlier
patent applications, filed in the last 12 months

Country

Priority application number
(if you know it)Date of filing
(day / month / year)7. Divisionals, etc: Complete this section only if
this application is a divisional application or
resulted from an entitlement dispute (see note f)

Number of earlier UK application

Date of filing
(day / month / year)8. Is a Patents Form 7/77 (Statement of
inventorship and of right to grant of a patent)
required in support of this request?

Yes

Answer YES if:

- a) any applicant named in part 3 is not an inventor, or
 - b) there is an inventor who is not named as an applicant, or
 - c) any named applicant is a corporate body.
- Otherwise answer NO (See note d)

Patents Form 1/77

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form

Description	42 ✓
Claim(s)	2 ✓
Abstract	
Drawing(s)	2 + 2 JMW

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for a preliminary examination and search (Patents Form 9/77)

Request for a substantive examination (Patents Form 10/77)

Any other documents
(please specify)

11.

I/We request the grant of a patent on the basis of this application

Mark Goddard
Signature

Date 4 June 2004

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

Chris Goddard
020 7206 0600

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- a) If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- b) Write your answers in capital letters using black ink or you may type them.
- c) If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s) of the form. Any continuation sheet should be attached to this form.
- d) If you have answered 'Yes', Patents Form 7/77 will need to be filed.
- e) Once you have filled in the form you must remember to sign and date it.
- f) Part 7 should only be completed when a divisional application is being made under section 15(4), or when an application is being made under section 8(3), 12(6) or 37(4) following an entitlement dispute. By completing part 7, you are requesting that this application takes the same filing date as an earlier UK application. If you want the new application to have the same priority date(s) as the earlier UK application, you should also complete part 6 with the priority details.

Compositions

The present invention relates to the protection, stabilisation and delivery of peptides and proteins in pharmaceutical and neutraceutical compositions. In 5 particular, the invention relates to compositions and formulations for delivering cationic poly- and oligopeptides in lipid based delivery systems so as to provide better protection against degradation by 10 peptidases and proteases.

There is an enormous potential in the use of peptides and proteins for treating various disease states, as well as in prophylaxis and in improving general health 15 and wellbeing of subjects. However, the performance of administered peptide agents is generally limited due to poor bioavailability, which in turn is caused by the rapid degradation of peptides and proteins in biological fluids. This increases the dose which must be 20 administered and in many cases restricts the effective routes of administration. These effects are further exaggerated by the often limited permeability of peptides and proteins across biological membranes.

Peptides and proteins that are administered to the 25 mammalian body (e.g. orally, intramuscularly etc.) are subject to degradation by various proteolytic enzymes and systems present throughout the body. Well known sites of peptidase activity include the stomach (e.g. pepsin), and the intestinal tract (e.g. trypsin, 30 chymotrypsin, and others) but other peptidases (e.g. the carboxypeptidases A, B & C) are found throughout the body. Upon oral administration, gastric and intestinal degradation reduces the amount of peptide or protein 35 which potentially could be absorbed through the intestinal surface lining and thereby decrease their bioavailability. Similarly, free peptides and proteins

in the mammalian blood stream are also subject to enzymic degradation (e.g. by plasma carboxy peptidases etc.).

5 A small number of the proteases (peptidases) found in the mammalian body are indicated in Table 1, along with their type and common sites of action.

Table 1

	PROTEASE	SOURCE	TYPE
10	Chymotrypsin A & B	Intestine	Serine protease
	Chymotrypsin C	Intestine	Serine protease
	Trypsin	Intestine	Serine protease
	Elastase	Intestine	Serine protease
15	Pepsin A	Stomach	Aspartic protease
	Carboxypeptidase A	Intestine	Zinc metallo protease
	Carboxypeptidase B	Intestine	Zinc metallo protease
	Carboxypeptidase C	Ubiquitous	Serine protease
	Cathepsin B	Lysosomes	Cysteine protease
20	Cathepsin C	Ubiquitous	Cysteine protease
	Cathepsin G	Polymorphonuclear leukocytes	Serine protease
	Cathepsin H	Lysosomes	Cysteine protease
	Cathepsin X	Ubiquitous	Cysteine protease
25	Aminopeptidase A	Ubiquitous, membrane bound	Zinc metallo protease
	Aminopeptidase B	Ubiquitous, cytosolic or membrane bound	Zinc metallo protease

Low permeability and absorption is another major obstacle leading to poor bioavailability of peptides in oral systems. Lipid carriers have in some cases been shown to significantly enhance the absorption of peptides and proteins but generally the (especially oral) bioavailability of peptide agents is so low as to render them essentially ineffective.

35 Two examples of cationic peptide active agents are the cyclic nonapeptide desmopressin and the 32-amino acid peptide calcitonin.

Calcitonin is a naturally occurring peptide hormone secreted by the parafollicular cells of the mammalian thyroid gland and is also found in birds and fish. From all origins, calcitonin is a 32-residue peptide with one disulphide bridge and although some residues are conserved universally, some calcitonins vary significantly in other parts of their primary sequence. Calcitonin is produced naturally in response to elevated plasma calcium levels and can be used in the control, treatment and prophylaxis of conditions such as Paget's disease of bone, hypercalcemia and post-menopausal osteoporosis.

The most commonly administered calcitonins for human use are synthetic human calcitonin, and synthetic salmon calcitonin (which is around 50 times as potent by weight as the human hormone). Calcitonin can be administered only by parenteral routes due partly to rapid degradation in the gastro-intestinal tract (GI tract). Usually, administration is intravenous (IV), intramuscular (IM) or subcutaneous (SC). This is necessary, but is undesirable because treatment with calcitonin is usually prolonged and a patient will typically have to self-administer the hormone by subcutaneous injection on a regular basis, often at alternating sites. As a result, an effective oral formulation would be a significant advance.

Desmopressin is a synthetic analogue of the natural human posterior pituitary hormone arginine vasopressin. Desmopressin is an antidiuretic and is used to prevent or control polydipsia, polyuria, and dehydration in patients with a deficiency in endogenous posterior pituitary vasopressin (e.g. in diabetes insipidus).

Unlike calcitonin, desmopressin may be administered orally due to high potency and limited side effects but

its oral availability remains extremely low. Nasal or intravenous administration are considerably more effective than ingestion, with nasal administration giving at least 20 times the bioavailability of the oral formulation and intravenous administration being more than 600 times as effective. Nonetheless, oral administration of desmopressin is highly preferred and it would be of considerable advantage to provide oral compositions of this peptide with greater bioavailability.

As illustrated in the above examples, there is a considerably need for a method by which peptide active agents may be protected from the proteolytic activity of the GI tract. There is also a need for a method by which peptides may be formulated for intramuscular or subcutaneous "depot" injection and remain protected from degradation within the body for longer periods of time.

The formulation of active agents with amphiphiles such as lipids has recently generated significant interest. Lipids have a polar, hydrophilic group and a non-polar hydrophobic group and these groups can assemble through mutual interactions into a variety of ordered and disordered structures in the presence of polar and/or non-polar solvents. Where the spontaneous curvature of the lipid is low, these structures are typically lamellar, such as mono- or multi-lamellar vesicles and liposomes and where the spontaneous curvature is higher, micellar phases or liquid crystalline phases dominate.

Amphiphile-based formulations show considerable potential in the delivery of many substances, especially for *in vivo* delivery to the human or animal body. Because the amphiphile has both polar and apolar groups which cluster to form polar and apolar regions, it can effectively solubilise both polar and apolar compounds.

In addition, many of the structures formed by amphiphiles/structuring agents in polar and/or apolar solvents have a very considerable area of polar/apolar boundary at which other amphiphilic compounds can be adsorbed and stabilised. Amphiphiles can also be formulated to protect active agents, to at least some extent, from aggressive biological environments and thereby provide advantageous rates and sites of active agent release.

10

The formation of non-lamellar regions in the amphiphile/water, amphiphile/oil and amphiphile/oil/water phase diagrams is a well known phenomenon. Such phases include liquid crystalline phases such as the cubic P, cubic D, cubic G and hexagonal phases, which are fluid at the molecular level but show significant long-range order, and the L₃ phase which comprises a multiply interconnected bi-continuous network of bilayer sheets which are non-lamellar but lack the long-range order of the liquid crystalline phases. Depending upon their curvature, these phases may be described as normal (mean curvature towards the apolar region) or reversed (mean curvature towards the polar region).

25

The non-lamellar liquid crystalline and L₃ phases are thermodynamically stable systems. That is to say, they are not simply a meta-stable state that will separate and/or reform into layers, lamellar phases or the like, but are the stable thermodynamic form of the mixture.

30

Both lamellar and non-lamellar systems have been investigated for their properties as carriers and/or excipients for dietary, cosmetic, nutritional, diagnostic and pharmaceutical agents but the non-lamellar systems are thought to have considerable advantages in terms of their high internal surface area

and bicontinuous polar and apolar regions. This has led to considerable investigation of non-lamellar phases particularly in controlled-release formulations and for solubilising relatively insoluble compounds.

- 5 As discussed above, the bulk non-lamellar phase is typically a thermodynamically stable system. In addition, this bulk phase may be dispersed in a polar or non-polar solvent to form particles of a non-lamellar 10 (especially liquid crystalline) phase in a bulk solvent. This allows the advantages of bulk non-lamellar phases to be applied in situations where use of a bulk formulation that was not miscible with a body fluid would cause problems, such as in parenteral 15 applications. Further control of a compound's release profile may also be achieved by, for example, optimising the size, morphology and drug-carrier membrane interactions of such dispersions.
- 20 In many cases, a liquid crystalline or L_3 phase is in or near thermodynamic equilibrium with the excess solvent and therefore stable dispersions of non-lamellar particles can be prepared. Such particles may be fully (i.e. thermodynamically) stable, or may gradually 25 degrade, thereby providing control over the release profile for active agents formulated therewith. The formation of dispersions can be spontaneous or as the result of mechanical force introduced by, for example, shearing or ultrasound. These non-lamellar particles 30 are of considerable interest in the delivery of active agents and have been proposed as carriers for many such actives.
- 35 A method for the formation of dispersed particles of non-lamellar phase in solvents such as water is described in US 5,531,925. Such particles have a non-lamellar liquid crystalline or L_3 interior phase, or

combinations thereof and typically a lamellar or L₃ surface phase.

Known particles of liquid crystalline or L₃ interior phase may be formed by methods such as adding to this phase a solution of surface-phase forming and stabilising agents, stirring to form a coarse dispersion and fragmenting the resulting mixture. Non-lamellar particles may also form spontaneously when dissolving a suitable composition of structure forming and fragmenting/stabilising agents in solvent.

In order to assess the presence of a liquid crystalline phase, the liquid crystalline order discussed above may be examined by use of small-angle X-ray diffraction (SAX), cryo-Transmission Electron Microscopy (cryo-TEM) or Nuclear Magnetic Resonance (NMR) spectroscopy studies. The sizes and size distributions of the dispersed particles may be examined by light scattering, particularly by use of laser light scattering or laser light diffraction instruments.

Emulsion, micellar or non-lamellar phase formulations are generally formed from mixtures containing at least one amphiphile with at least one hydrophilic "head" group and at least one hydrophobic "tail" group. In most naturally occurring lipids, these groups are joined by an ester linkage with the "tail" consisting of the hydrocarbon chain of the fatty acid(s). Typically, therefore, amphiphile-containing preparations of active agents contain either natural ester-containing lipids, synthetic (i.e. not found in natural extracts) amphiphiles or mixtures thereof.

The present inventors have now unexpectedly established that the lifetime of cationic peptides upon oral administration or subcutaneous or intramuscular

injection may be considerably increased by formulation of the cationic peptides in a non-lamellar formulation wherein the formulation comprises an anionic amphiphile, particularly a fatty acid.

5

In a first aspect, the present invention therefore provides a composition comprising at least one cationic peptide active agent, at least one neutral structure forming amphiphile, at least one anionic structure forming amphiphile and optionally at least one solvent wherein said composition comprises a non-lamellar phase structure and/or forms a non-lamellar phase structure on exposure to body fluids. Preferably, the composition will be suitable for administration to a mammalian body such as a human, canine, feline, bovine, equine, porcine, caprine or ovine body, most preferably a human body. Furthermore, the non-lamellar phase structure adopted or formed by the composition will preferably be non-lamellar phase particles.

10

The present invention results in a considerably higher oral bioavailability in mammalian subjects than has previously been achievable. In particular, this can be seen as a higher proportion of the administered dose being detectable in the bloodstream, relative to the amount that would be present if the same dose were administered directly (e.g. intravenously in saline solution).

15

20 In a second aspect, the invention therefore provides a composition comprising at least one cationic peptide active agent, at least one neutral structure forming amphiphile, at least one anionic structure forming amphiphile and optionally at least one solvent wherein said composition comprises particles of non-lamellar phase and/or forms particles of non-lamellar phase on exposure to body fluids and wherein the oral

25

bioavailability of said active agent is at least 1% when measured as blood plasma concentration of active agent relative to intravenous administration in saline solution.

5

In a further aspect, the invention also provides a method for the formation of a composition of the invention comprising forming particles of non-lamellar phase and/or particles which generate non-lamellar phase 10 on exposure to body fluids, said particles comprising at least one neutral structure forming amphiphile, at least one anionic structure forming amphiphile or salt thereof and optionally at least one solvent, and subsequently contacting said particles with a solution of cationic 15 peptide active agent. The method may also comprise the step of drying the resulting peptide-containing dispersion, for example by freeze drying or spray drying.

20

Without being bound by theory, it is believed that the increased bioavailability of the cationic peptides administered as compositions of the present invention is due at least in part to reduced enzymic degradation. In particular, the inventors have shown that not only do 25 non-lamellar particulate formulations provide significant protection for peptides against proteolytic enzymes, but also that such non-lamellar formulations comprising anionic amphiphiles, and particularly fatty acids, are significantly more effective in such 30 protection than are other formulations.

The action of proteolytic enzymes such as trypsin in degrading cationic peptides has been shown to be significantly reduced by formulations comprising fatty acids according to the present invention. This reduction in enzymic degradation results in a greater proportion of the peptide active agent reaching its site

35

of absorption and crossing into the blood stream. It has been confirmed in animals that the level of peptide absorbed according to the present invention is noticeably higher than in the absence of these protective effects (see examples below).

In the present invention, the peptide active agents are generally cationic peptides or proteins and may range from small oligomers (such as di-, tri- or tetra-peptides) to large proteins with a molecular weight of many thousands of Daltons. All are referred to herein as "peptides" regardless of their secondary or tertiary structure. The peptides referred to herein will, however not generally be lipoproteins unless specifically indicated. Similarly, the terms "protease" and "peptidase" are used herein as equivalent unless otherwise indicated.

Peptides may consist purely of the 20 common α -amino acids of the "genetic code" (and optionally their stereoisomers) or may contain at least one other naturally occurring or non-naturally occurring amino acid unit such as β -, γ -, or δ -amino acids or the substituted equivalents of any of these. Common substituents include hydrocarbyl substituents (e.g. alkyl, alkenyl, alkynyl, aryl, aralkyl, or alkaryl groups), heterocyclic substituents (e.g. nitrogen, oxygen or sulphur containing mono- or polycyclic substituents), nitrogen-based substituents (e.g. amino, alkyl amino, nitro, urea or azo substituents), sulphur-based substituents (e.g. thio, thioalkyl, or sulphonyl substituents), halogen-based substituents (e.g. fluoro, chloro, bromo or iodo, chlorate, iodate etc.), oxygen-based substituents (such as keto, carboxy, ester, hydroxy or aldehyde, peroxide, ether substituents) and combinations thereof. Obviously, certain natural and non-natural substituents are capable of

5 bridging/crosslinking and frequently disulphide, ester, amide or other bridges may be present. Such crosslinks will reduce the conformational freedom of the peptides and frequently provide a more pronounced biological effect.

10 The formulation of the various components in the present invention may be carried out by known methods using the indicated amphiphilic components and peptide active agents. Suitable methods include those indicated in the present Examples and in US 5,531,925, WO 02/02716, WO 02/068561, WO 02/066014 and WO 02/068562. The disclosures of these and all references cited herein are hereby incorporated herein by reference.

15 Dispersion methods include adding liquid crystal phase forming amphiphiles (with or without fragmentation agents) in dry, molten or solution form, to an aqueous solution (optionally including fragmentation agents and 20 optionally including a lipid, such as phosphatidyl choline - PC) and either allowing natural fragmentation of the mixture or accelerating the process with, for example, mechanical agitation, vortexing, roto-stator mixing, high-pressure homogenation, microfluidisation 25 and/or ultrasound.

30 The phase behaviour and size distribution of particulate formulations in the invention may also be controlled by one or more (preferably one) cycles of heating and cooling. Such cycles can be used to convert lamellar particles to non-lamellar form, and to reduce the spread of particle sizes. Where this method is used, the compositions should, preferably, be formulated such that the thermodynamically stable state is non-lamellar. 35 Where heat cycling is used, the peptide active agent may be incorporated into the particles prior to and/or after heat cycling. Where more than one heat cycle is used,

the active agent may also or alternatively be incorporated between cycles. Since peptide active agents are often heat sensitive, the active agent is preferably incorporated only after any heat cycling is complete.

A heat cycle brings the composition, with or without the active agent present, up to a temperature sufficient to provide conversion of at least a portion of the particles to non-lamellar phase upon cooling to ambient temperature. This will typically involve heating to around 90-150°C for 1-30 min followed by cooling to ambient temperature. More typically a heat cycle will involve heating to 100-120°C for 2-20 minutes before cooling. The most suitable conditions will vary in detail between compositions but will be readily established by the skilled worker.

In the heat cycling process, the mean particle size typically increases but the distribution of particle sizes is reduced.

The presence of particles in non-lamellar form will preferably be assessed from a set of cryo-transmission electron microscopy particle images, preferably showing a sample of more than 50, preferably more than 100 particles. The presence of non-lamellar particles may also be assessed by X-ray scattering experiments.

The term "cationic" is used to indicate that the peptide active agents carry a net positive charge at the pH of the composition and/or at physiological pH. Generally, where the 20 amino acids commonly found in natural peptides and proteins dominate, a cationic peptide will have a greater number of basic amino acids such as lysine or arginine than acidic residues such as glutamic acid or aspartic acid.

In order to allow peptides not having an excess of basic functional groups to be used in the present invention, these peptides may be synthetically rendered "cationic", for example by protecting acid functional side-chains so 5 that these cannot ionise. By this method, the charge-balance may be altered to provide peptide prodrugs with a net positive charge.

Where a potentially negatively charged group such as a 10 carboxylic acid is protected, the protecting group should not affect the biological activity or should preferably be cleavable at the target site and should release a molecule with acceptable physiological properties. Ester and amide groups may both be used to 15 prevent the ionisation of carboxylic acid moieties, for example, and both are cleavable chemically or enzymically within the body. It would also be preferable, for example, to protect an acid group as the ethyl or iso-propyl ester, so as to generate 20 biologically tolerable ethanol or isopropanol upon deprotection, rather than to use a methyl ester which would generate undesirable methanol.

In a similar way to the above, additional positive 25 charge may be added to a peptide not having a sufficient cationic nature by provision of additional amine, or other groups having a positive charge at an appropriate pH. A serine or other hydroxy-containing residue might, for example, be esterified to the carboxy group of an 30 amino-acid (especially a natural amino acid such as glycine or alanine) so that the amino group provides an additional basic moiety. A similar reaction may be carried out with a free thiol group of a cystine, which might be attached by means of a disulphide bond to an 35 amino thiol. As with the protection of acid groups, the "activation" of neutral groups in this way will preferably be by means of bonds which may be cleaved

(e.g. enzymatically) at the target site so as to release molecules which are biologically tolerable (such as glycine or alanine in the above example). Methods for forming many suitable substitutions are well known in the art. References herein to peptide active agents also refer to their "prodrugs" such as those described above, unless otherwise indicated.

The cationic peptide active agent will typically be of pharmaceutical or veterinary activity (such as a therapeutic, a vaccine, a prophylactic or a diagnostic agent) but may also be a dietary or cosmetic agent or a general stimulant or immune-boosting agent. More than one cationic peptide active agent may be incorporated if appropriate and additional active agents, including peptides and non-peptides, may be incorporated into the formulations as appropriate. Any additional active agent may be present to reinforce the activity of the cationic peptide active agent or may be provided to, for example, further increase the uptake of the cationic peptide or to suppress one or more undesirable effects of the cationic peptide active agent. In one preferred embodiment of the invention, the cationic peptide active agent is formulated in accordance with the invention in the presence of at least one protease inhibitor. By formulation with a protease inhibitor, the protective effect of the invention is further strengthened and a still greater proportion of the peptide active agent is delivered. Suitable protease inhibitors include pepsin inhibitors, trypsin inhibitors and chymotrypsin inhibitors, such as Trasylol® (aprotinin), amastatin, leupeptinebovine pancreatic trypsin inhibitor, ecotin, Streptomyces pepsin inhibitor, pepstatin, acetic acid chymotrypsin inhibitor I etc.

Generally, the cationic peptide active referred to in the present invention will be "enzyme sensitive" or

"peptidase sensitive". As used herein, this indicates that at least one of the peptide bonds forming the peptide is susceptible to cleavage by proteolytic enzymes. Sensitivity to such enzymes may be measured by standard *in vitro* methods which will be familiar to those of skill in the art. Such methods are similar to those indicated in the Examples below for *in vitro* testing of the stabilising effect of the present invention. Typically, the peptides in the present invention will be sensitive to at least one peptidase and/or protease occurring in the mammalian gastro-intestinal tract such as trypsin, carboxypeptidase C, chymotrypsin, or pepsin. Preferably, the cationic peptide active agents are carboxypeptidase C sensitive.

As use herein, the term "non-lamellar" is used to indicate a normal or reversed liquid crystalline phase (such as a cubic or hexagonal phase) or the L₃ phase or any combination thereof. Where a particle is described as having a non-lamellar phase or form, this indicates that amphiphiles in at least the internal region of the particle should adopt this form. The particles will generally have two distinct regions, an internal region and a surrounding surface region. The surface region, even in a "non-lamellar" particle will typically be lamellar, L₃ or crystalline. In contrast, a "lamellar" particle, as described herein is a particle having a solvent, rather than non-lamellar, core-region. Preferred non-lamellar forms are reversed liquid crystalline phases such as cubic or hexagonal phases. A highly preferred liquid crystalline phase is the reversed hexagonal phase.

A composition is considered "non-lamellar" if a molecular fraction of at least 30% of the structure forming amphiphile exists as non-lamellar phase particles. Similarly, a composition forms non-lamellar

phase particles if at least 30% of the amphiphile is in the form of such phases after exposure to an aqueous fluid. This will generally be at least 50% in both cases and preferably at least 70% of the amphiphilic component should be in a non-lamellar form, either in the composition as formulated or after exposure to a body fluid. More preferably this is at least 80%, most preferably 90% or more.

10 Compositions which form non-lamellar phases on exposure to body fluids, (compositions precursors) will generally contain amphiphilic, active peptide and optionally fragmentation components in similar relative proportions to the non-lamellar dispersions themselves but will

15 typically have a lower proportion of the optional solvent. This solvent is generally aqueous or miscible with water and the composition precursors may be formed by drying non-lamellar compositions, for example by spray-drying or freeze drying. The precursors may also 20 contain components such as sugars (e.g. lactose) to help stabilise and protect the compositions upon drying and/or to aid rehydration. Water miscible solvents are typically biologically acceptable and include alcohols (especially ethanol and isopropanol), glycerol,

25 ethylene/propylene glycol (especially oligo-ethylene and/or propylene glycol) and short-chain mono glycerides (e.g. up to C₆, especially straight chain propyl, butyl, pentyl or hexyl optionally with one or more unsaturations).

30 Dispersions containing active ingredients and particularly those for intravenous administration to the human or animal body are desirably colloidal, that is they should be of a particle size no greater than 10 μm, especially no greater than 5 μm and particularly no greater than 1 μm. If particles within the dispersion exceed this size then the dispersion may not be

colloidally stable and there is a considerable risk of causing embolism when the preparation is administered intravenously. Furthermore, it is desirable that the distribution of particle sizes be narrow to maximise
5 control over the release of any active agent.

In the present case, compositions will often be administered by a method other than intravenously (especially orally, intramuscularly or subcutaneously),
10 and thus the particle size need not be colloidal. In such cases, typical particle sizes range from around 10 µm to around 200 µm. It remains, however, advantageous for particulate compositions to provide well characterised and reproducible particle size
15 distributions in order to control the rate of decomposition of the particles and/or release of the active agents.

The "structure forming amphiphile" as indicated herein includes any agents that are capable of forming a structured phase in the presence of an aqueous solvent, optionally in the presence of other agents such as other amphiphiles and/or fragmentation agents. The amphiphiles will have at least one polar, hydrophilic group and at least one non-polar, hydrophobic group.
25

Examples of polar groups are well known (see e.g. US published patent application number 20020153509) and include anionic groups such as carboxylates,
30 phosphonates, sulphates and sulphonates, non-ionic groups such as alcohols, polyols (eg sugars, glycerol etc) and esters, cationic groups such as quaternary ammonium compounds, pyridinium salts and quaternary phosphonium salts and zwitterionic groups such as
35 phospholipid head groups (e.g phosphatidyl-choline etc.), ammonioacetates, ammonio-alkanesulphonates and trialkylaminoalkylphosphate esters. The neutral

amphiphile component will have no net charge at the pH of the composition and/or physiological pH while the anionic lipid component will carry a net negative charge under such conditions. Preferably, the neutral 5 amphiphile component will be non-ionic. Furthermore, the anionic lipid will preferably include at least one acid group, or salt thereof, particularly a carboxylic acid group. Cationic amphiphilic components are not preferred and where present, there will be a greater 10 quantity of anionic amphiphiles than cationic amphiphiles.

Examples of non-polar groups include C₆-C₃₂ alkyl and alkenyl groups, which are typically present as the 15 esters of long chain carboxylic acids. These are often described by reference to the number of carbon atoms and the number of unsaturations in the carbon chain. Thus, CX:Z indicates a hydrocarbon chain having X carbon atoms and Z unsaturations. Examples particularly include 20 caproyl (C₆:0), capryloyl (C₈:0), capryl (C₁₀:0), lauroyl (C₁₂:0), myristoyl (C₁₄:0), palmitoyl (C₁₆:0), phytanoyl (C₁₆:0), palmitoleoyl (C₁₆:1), stearoyl 25 (C₁₈:0), oleoyl (C₁₈:1), elaidoyl (C₁₈:1), linoleoyl (C₁₈:2), linolenoyl (C₁₈:3), arachidonoyl (C₂₀:4), behenoyl (C₂₂:0) and lignoceroyl (C₂₄:9) groups. An 30 amphiphile will typically have one or two non-polar "tail" groups (mono-acyl and di-acyl lipids respectively) but may have three, four or more hydrophobic groups.

Examples of amphiphiles suitable for use in the present invention include natural lipids, synthetic lipids, 35 surfactants, copolymers, proteins (in particular caseins and albumin), hydrotropes, alcohols, and other additives that may form or facilitate formation of structured phases. Preferred agents are glycerides (e.g. monoglycerides, diglycerides, and triglycerides), di-

and polyglycerolesters of glycerides (e.g. diglycerol monooleate, diglycerol monocaprate), natural fats and oils (e.g. soybean oil, coconut oil, corn oil, castor oil, sunflower oil), fractionated oils (e.g. 5 fractionated coconut oil, Miglyol® (Condea)), transesterified oils (e.g. Maizine®), transesterification products of oils and PEG (e.g. ethoxylated castor oil (e.g. Cremophor® EL (BASF)), ethoxylated hydrogenated castor oil (e.g. Cremophor® 10 RH-40 (BASF)), ethoxylated corn oil (e.g. Labrafil® M 2125 CS (Gattefossé))), acetylated monoglycerides, fatty acids (e.g. C6-C26 saturated and unsaturated fatty acids), fatty alcohols (e.g. phytantriol (3,7,11,15-tetramethyl-1,2,3-hexadecantriol)), ether lipids (e.g. 15 monooleyl glyceryl ether), natural and synthetic phospholipids (e.g. egg lecithin, soya lecithin, hydroxylated lecithin, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl glycerol, phosphatidic acid), 20 lysophospholipids (e.g. lyso-lecithin, lyso-phosphatidyl choline, lyso-oleyl phosphatidyl choline), phospholipid-analogous compounds (e.g. those disclosed in US 6344576), sterols and sterol derivatives (e.g. cholesterol, sitosterol, lanesterol and their esters, 25 especially with PEG or fatty acids), galactolipids (e.g. digalactosyl diacylglycerol, monogalactosyl diacylglycerol), sphingolipids (e.g. sphingomyelin); nonionic surfactants, in particular ethoxylated surfactants such as PEG-fatty acid mono- and diesters 30 (e.g. of the Crodet® (Croda), Cithrol® (Croda), Nikkol® (Nikko), Myrj® (ICI) series, Solutol® HS 15 (BASF)), PEG glycerol fatty acid esters (e.g. Tagat® L and O (Goldschmidt); Glycerol® L series (Croda), Capmul® EMG (Abitec)), transesterification products of oils and PEG 35 (e.g. of the Labrafil® (Gattefossé), Cremophor® (BASF) Crovol® (Croda) and Nikkol® HCO (Nikko) series), PEG-sorbitan fatty acid esters (e.g. Tween® 20, Tween®

- 80 and other polysorbates of the Tween® series (ICI)), PEG alkyl esters (e.g. of the Brij® (ICI) and Volpo® (Croda) series), PEG alkyl phenol surfactants (e.g. of the Triton X and N series (Rohm & Haas); polyglycerised fatty acids (e.g. Nikkol® Decaglyn (Nikko), Plurol® Oleique (Gattefossé)), propylene glycol fatty acid esters), propylene glycol fatty acid esters (e.g. Capryol® 90 (Gattefossé), Lutrol® OP2000 (BASF), Captex® (Abitec)), glycerol/propylene glycol fatty acid esters (e.g. Arlacel® 186 (ICI)), sorbitan fatty acid esters (e.g. of the Span® (ICI) and Crill® (Croda) series), sugar esters (e.g. of the SUCRO ESTER® (Gattefossé) and Crodesta® (Croda) series), polyoxyethylene-polyoxypolypropylene block copolymers (so-called poloxamers, e.g. of the Pluronic® (BASF), Synperonic® (ICI) and Lutrol® (BASF) series), copolymers of ethylene oxide and butylene oxide; anionic surfactants including fatty acid salts, bile salts (e.g. sodium cholate, sodium glycocholate, sodium taurocholate), carboxylates such as ether carboxylates, succinylated monoglycerides, mono/diacetylated tartaric acid esters of mono- and diglycerides, citric acid esters of mono- and diglycerides, glyceryl-läcto esters of fatty acids, acyl lactylates, alginate salts, propylene glycol alginate; cationic surfactants including ethoxylated amines (e.g. polyoxyethylene-15 coconut amine), betaines (e.g. N-lauryl-N,N-dimethylglycine), alkylpyridinium salts, quarternary ammonium salts such as hexadecyl triammonium bromide, decyl trimethyl ammonium bromide, cetyl trimethyl ammonium bromide; zwitterionic surfactants including trimethylammonioethylalkylphosphonates (e.g. the examples disclosed in US 6344576); and all mixtures thereof.
- The most preferred neutral structure-forming agents are glyceryl monooleate, glyceryl monolinoleate, glyceryl dioleate (GDO), dioleyl phosphatidyl ethanolamine

(DOPE), dioleyl phosphatidylcholine (DOPC) and phytantriol, or lyso-phospholipids, especially lyso-oleyl phosphatidylcholine (LOPC). Any mixtures of these, particularly including GDO/DOPC or GDO:Egg 5 lecithin (e.g. 70:30 wt/wt of either) are also suitable. Naturally occurring lipids from natural or synthetic sources are preferred due to their generally lower and/or more predictable toxicity profile. Naturally occurring fatty acid ester lipids including phospholipids 10 are highly suitable.

Often the amphiphilic component will contain material in the form of extracted and purified natural products and will thus contain a mixture of related compounds. Soy 15 bean phosphatidyl choline, for example, is a mixture of compounds having around 60-75% C18:2 acyl groups, around 12-16% C16:0 and the balance others. Similarly, commercial egg lecithin is typically around 70-75% phosphatidyl choline, around 10% phosphatidyl 20 ethanolamine and the balance other lipids. Both of these products are suitable for use in the present invention. Different commercial preparations will also vary slightly but remain suitable.

An example preferred structuring agent for use in the present invention is commercially available glyceryl 25 monooleate (GMO). As indicated above, this is largely monoglyceride with an oleoyl (C18:1) acyl chain but contains certain amounts of other compounds. These are included in the term "glyceryl monooleate" or "GMO" as 30 used herein. Commercial preparations of GMO include GMOrphic-80 and Myverol 18-99 (available from Eastman Kodak), Rylo MG 19 and Dimodan DGMO (available from Danisco). Any of the structure-forming amphiphiles may 35 be used alone or in combination with one or more other amphiphilic structuring agents.

A key component in the present invention is the anionic lipid component as this provides unexpectedly high levels of protection for the peptide active agent against enzymic degradation. Any anionic amphiphiles or combinations thereof may be used, including those indicated above, but it is preferred to use at least one fatty acid or fatty acid salt component. Preferred fatty acids are those corresponding to the fatty acid chains of natural ester lipids, including caproic, 5 caprylic, capric, lauric, myristic, palmitic, phytanic, palmitolic, stearic, oleic, elaidic, linoleic, 10 linolenic, arachidonic, behenic or lignoceric acids, their salts or mixtures thereof. The salts of fatty acids will be physiologically tolerable salts. The most 15 preferred anionic amphiphiles are unsaturated naturally occurring fatty acids and their salts, especially oleic acid or salts thereof.

Preferred salts of any of the anionic components 20 indicated herein, particularly the anionic amphiphiles, include alkali and alkaline earth metal salts as well as ammonium and alkylammonium salts. Preferred examples of these include sodium, potassium, lithium, calcium or magnesium salts, ammonium salts or triethylammonium 25 salts. Where an anionic component or an acid is indicated herein, that indication refers also to physiologically tolerable salts thereof, unless specifically stated otherwise.

The anionic component will be present at a sufficient 30 concentration to increase the bioavailability of the cationic peptide. Very high concentrations of anionic amphiphiles such as fatty acids are not always desirable, however, from the point of view of other 35 important performance criteria including biocompatibility, dispersability, morphology and colloidal stability, as well as the health of the

subject. Thus, in some embodiments, the anionic component will be present in a quantity not significantly greater than necessary to provide the desired level of enzyme protection. Generally the 5 anionic component will be present in a concentration sufficient to increase the half-life of the cationic peptide in carboxypeptidase C solution *in vitro* by at least 50% relative to the composition in the absence of the anionic component. More preferably, this half-life 10 will increase by at least 75% or 100% and most preferably, will be 2.5, 3 or 4 times the value measured in the absence of anionic amphiphile.

15 The concentration of anionic amphiphile necessary will be readily determinable by simple experimentation, particularly with reference to the Examples herein and up to at least 50% anionic component may be used, as indicated below. Typically, however, the proportion of 20 anionic amphiphile to neutral amphiphile will be 0.5-20% w/w concentration range and more preferably between 1-10%w/w. The most preferred range is between 2 and 8% w/w.

25 Preferably the particulate dispersions referred to herein contain at least one fragmentation agent. The fragmentation agent serves to improve the dispersability of the non-lamellar phase; forms a stabilising phase around the non-lamellar particle and/or stabilises the dispersion. Suitable fragmentation agents will be agents 30 which aid the dispersal of amphiphile into particles (especially non-lamellar phase particles) or stabilise such particles. Typically a fragmentation agent will be a surfactant such as an amphiphilic block copolymer.

35 Important fragmentation agents include natural lipids, synthetic lipids, surfactants, copolymers, proteins (in particular caseins and albumin), hydrotropes, alcohols

and other additives that may facilitate fragmentation spontaneously or with the aid of externally applied forces and pressures and contribute to stabilisation. This includes also nanoparticles and combinations of 5 polymer and nanoparticles (see e.g. WO 99/12640).

Preferred fragmentation agents are copolymers and these 10 may have blocks comprising polyoxyalkylenes, polyvinylpyrrolidone, polyvinylacetate, polyvinylalcohol, polyesters, polyamides and/or polyalkenes. The block copolymer will comprise at least two blocks of polymer having different degrees of hydrophilicity. Certain proteins (such as casein) are also of amphiphilic character and may be used as 15 fragmentation agents. Where the cationic peptide active agent is an amphiphilic protein, this may act as both the active agent and the fragmentation agent, or may be included in addition to another active agent and/or fragmentation agent.

Preferred examples of amphiphilic block copolymers are 20 poloxamers, which comprise at least one block of polyoxyethylene and at least one block of polyoxypropylene. The most preferred fragmentation 25 agents are poloxamer 407 (e.g. Pluronic® (Lutrol) F127, BASF), poloxamer 188 (e.g. Pluronic® F68, BASF), and polysorbate 80 (e.g. Tween® 80, ICI).

Where included to aid dispersion, the fragmentation 30 agent will be present at a level sufficient to bring about the fragmentation of the composition and/or to stabilise the fragmented particles (which will preferably be non-lamellar phase). Such fragmentation may be spontaneous or may require physical fragmentation 35 such as by sheering and/or ultrasonication. It is preferable that sufficient fragmentation agent is present that the composition is physically stable.

Typically a fragmentation will provide a desired effect at a level of 1-30% by weight, relative to the total amphiphile content of the composition. This will more typically be 5-15% by weight and most preferably 8-12%
5 by weight.

Active agents suitable for the present invention are natural and synthetic cationic peptides and include human and veterinary drugs and vaccines, diagnostic
10 agents, cosmetic agents, nutrients, dietary supplements etc. These may be inherently cationic or may be rendered cationic by appropriate synthetic protection or activation as discussed supra. They may also comprise non-natural amino acids as indicated above.
15

Examples of suitable drugs include antibacterial agents including macrocyclic peptide antibiotics, anti fungal agents, anticancer and/or anti viral agents, anti inflammatory, cardiovascular drugs including
20 cholesterol lowering and blood-pressure lowing agents, analgesics, antidepressants, hormones, vaccines and bone modulators. Diagnostic agents include radionuclide labelled compounds and contrast agents including X-ray enhancing agents such as iodinated compounds and gamma-
25 radiation emitting compounds. Nutrients include dietary supplements etc.

Preferred active agents include human and veterinary peptide drugs selected from the group consisting of
30 adrenocorticotrophic hormone (ACTH) and its fragments, angiotensin and its related peptides, antibodies and their fragments, antigens and their fragments, atrial natriuretic peptides, bioadhesive peptides, Bradykinins and their related peptides, calcitonins and their
35 related peptides, cell surface receptor protein fragments, chemotactic peptides, cyclosporins, cytokines, Dynorphins and their related peptides,

endorphins and P-lidotropin fragments, enkephalin and their related proteins, enzyme inhibitors, fibronectin fragments and their related peptides, gastrointestinal peptides, growth hormone releasing peptides,
5 immunostimulating peptides, interleukins, lutenizing hormone releasing hormones (LHRH) and their related peptides, melanocyte stimulating hormones and their related peptides, nuclear localization signal related peptides, neuropeptides and their related peptides,
10 neurotransmitter peptides, opioid peptides, oxytocins, vasopressins and their related peptides (especially desmopressin); parathyroid hormone and its fragments, protein kinases and their related peptides,
15 somatostatins and their related peptides, substance P and its related peptides, transforming growth factors (TGF) and their related peptides, tumour necrosis factor fragments, toxins and toxoids and functional peptides such as anticancer peptides including angiostatins, antihypertension peptides, anti-blood clotting peptides,
20 and antimicrobial peptides; selected from the group consisting of proteins such as immunoglobulins, angiogenins, bone morphogenic proteins, chemokines, colony stimulating factors (CSF), cytokines, growth factors, interferons, interleukins, leptins, leukemia inhibitory factors, stem cell factors, transforming growth factors and tumour necrosis factors, all
25 derivatised as necessary to render them cationic.

Most preferably, the cationic peptide active agent will
30 be inherently cationic, in that it will have a net positive charge at the pH of the formulation, without any chemical modification.

Some examples of peptide active agents, along with an
35 outline of some of their properties and/or target indications is given below in Table 2. The table also shows the amino acid number and isoelectric point of

certain peptides.

Preferred peptide active agents are cationic and this may be measured by the isoelectric pH of the peptide, e.g. in its active form and/or when modified as a prodrug (as described above). At a pH below the isoelectric value, a peptide can be considered cationic. Thus, preferred peptides will have an isoelectric point of at least 6.5, especially at least 7.0 and most preferably 8 or more (e.g at least 8.5 or at least 9.0). Calcitonin, for example has its isoelectric point at around pH 8.9 and vasopressin at around 8.0. Examples of all such peptides are given in Table 2. Correspondingly, the peptide active agents are preferably not peptides such as lepuridin or insulin α , which have a isoelectric values of around 4.

Table 2 - Peptide and Protein Active Agents

20	1a HORMONES & HORMONE DERIVATIVES	Amino Acids	Isoelectric pH
	Somatotropin Growth hormone deficiency and for Turner's syndrome	191	5.27
25	Somatostatin Acromegaly and carcinoid and vasoactive intestinal peptide tumours	28	9.58
	Calcitonin (salmon) Osteoporosis	32	8.86
	Oxytocin induction of birth labour & maintenance of milk secretion	9	5.51
30	Gonadorelin Ovulation control <i>Derivatives:</i> Leuprolide; Goserelin; Triptorelin	10	8.75
	Vassopresin Diabetes insipidus, haemorrhaging esophageal varices, etc. <i>Derivatives:</i> Desmopressin, Felypressin	9	8.06
35	Follitropin-beta Infertility	111	5.91
	Follitropin-alpha Infertility	116	8.38
40	human Chorionic Gonadotropin-beta Infertility	145	8.65

	Thyrotropin alpha adjunctive diagnostic tool for serum thyroglobulin Secretin (e.g. swine) pancreatic assessment Bradykinin hypotensive tissue hormone Insulin α Insulin β	92 27 9 21 30	8.38 9.45 3.79 6.9
5			
10	1b ANTIVIRAL, ANTIBACTERIAL & ANTIFUNGAL	Amino Acids	Isoelectric pH
	Interferon-alpha 1/13 Interferon-alpha 2 Interferon-beta Interferon-gamma	166 165 166 166	5.18 5.99 8.93 9.54
15	In different recombinant forms, anti- hepatitis C, leukemia, sclerosis Tachyplesin I antibacterial, antiviral Tuftsin immunomodulator, antimicrobial, antiviral, anticancer	17 4	9.93 11
20	Magainin I Magainin II Inhibit the growth of numerous bacteria and fungi Indolicidin (e.g. bovine) antibacterial, antifungal, anticancer, antiviral, antiparasite	23 23 13	10 10 12.01
25	Protegrin (e.g. swine) antibacterial, antifungal, antiviral Polyphemusin I Polyphemusin II antibacterial, antifungal, antiviral	18 18 18	10.66 10.33 10.1
30	Polymixin B antibacterial Gramicidin S antifungal	10	
35	1c OTHER PEPTIDES & PROTEINS	Amino Acids	Isoelectric pH
	Intercellular Adhesion Molecule 1 Pneumadin antidiuretic Alteplase	23 10 527	9.51 4.36 7.61
40	Acute myocardial infarction and acute ischemic stroke Interleukin-1 receptor antagonist anti rheumatoid arthritis GMCSF Filgrastim (G-CSF)	550 127 177	8.51 5.21 5.43

	neutropenia			
5	Lepirudin	65	4.04	
	anticoagulant			
	Becaplermin	109	9.38	
	Diabetic foot ulcers			
	OspA	257	6.72	
	Prevention of Lyme disease			
10	Avicine	37	4.58	
	Anticancer			
10	Tubulysins A-F			
	anticancer			
	Contakulin G (CGX-1160)			
	analgesic			
15	alpha conotoxin-like peptides	16		
	analgesic (see WO 02/079236)			
	Mellitin	26	12.02	
	analgesic, antibacterial			
	1d INTERLEUKINS (ILs)	Amino Acids	Isoelectric pH	
20	IL-1alpha	159	5.3	
	Hematopoietin-1			
	IL-1 beta	153	5.91	
	Catabolin			
25	IL-2	133	7.05	
	T-cell growth factor (TCGF) (Aldesleukin).			
	IL-3	133	6.97	
	Haematopoietic growth factor			
	IL-4	129	9.26	
30	B-cell stimulatory factor			
	IL-5	115	7.02	
	T-cell replacing factor			
	IL-6	183	6.21	
	B-cell stimulatory factor			
35	IL-7	152	8.72	
	IL-8	77	9.24	
	Neutrophil-activating			
	IL-9	126		
40	T-cell growth factor P40			
	IL-10	160	7.63	
	Cytokine synthesis inhibitory factor			
	IL-11	178	11.16	
	Adipogenesis inhibitory factor			
	IL-13	112	8.81	
45	IL-15	114	4.52	

	IL-17	132	8.62
	Cytotoxic T lymphocyte-associated antigen 8	157	5.04
5	IL-18	153	7.8
	Interferon-gamma inducing factor	152	8.77
	IL-19	158	8.6
	Melanoma differentiation associated protein-like protein	150	9.99
10	IL-20		
	Four alpha helix cytokine ZCYTO10		
	IL-24		
	Melanoma differentiation associated protein 7		
	IL-26		

Preferred examples from this list include Somatostatin,
15 Calcitonin (salmon), Gonadorelin, Vassopresin,
Follitropin-alpha, human Chorionic Gonadotropin-beta
hCG-beta, Thyrotropin alpha, Secretin, Bradykinin,
Interferon-beta, Interferon-gamma, Tachyplesin I,
Tuftsin, Magainin I, Magainin II, Indolicidin,
20 Protegrin, Polypheinisn I, Polypheinisn II, Polymixin B,
Gramicidin S, Intercellular Adhesion Molecule 1,
Alteplase, Interleukin-1 receptor antagonist,
Becaplermin, Tubulysins A-F, Contakulin G (CGX-1160),
alpha conotoxin-like peptides, Mellitin, IL-2, IL-3,
25 IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-13,
IL-17, IL-19, IL-20, IL-24 and IL-26. The most
preferred cationic peptide active agents are the
inherently cationic peptides calcitonin (human or
preferably salmon) and desmopressin.

30 Compositions of the present invention may be formulated
as pharmaceuticals by methods well known in the art.
These formulations will typically be oral formulation
such as tablets, coated tablets (such as controlled
release tablets), capsules, suspensions, dispersions,
35 syrups, or powders, but may be formulations for
inhalation (such as powders or aerosols), or for
parenteral (e.g subcutaneous, intramuscular or
intravenous) administration in the form of, for example,

sterile dispersions in saline, or precursors thereof. One particularly interesting embodiment relating to parenteral "depot" administration is described in detail below.

5

The compositions may be formulated with conventional pharmaceutical carriers, diluents and/or excipients such as aqueous carriers (e.g. water for injections), solvents, binders, fillers, stabilizers, osmolality adjusting agents, effervescing agents, pH buffers and modifiers, viscosity modifiers, sweeteners, lubricants, emulsifiers, flavours, coating agents (e.g. gastric juice resistant coatings) etc. Formulations comprising at least one pharmaceutically acceptable carrier and/or diluent thus form a further preferred aspect of the invention.

20 The dosage of the compositions of the invention to be administered to a subject will be dependent upon the active agent, the species, size, maturity, health and condition of the subject and upon the formulation chosen. The compositions may also deliver a larger proportion of the active agent to the subject than traditional formulations and this should be taken into account. Suitable dosages will be readily established for known therapeutic peptides by reference to the bioavailability when formulated in the compositions of the invention and the known dosage and bioavailability when delivered by established methods.

25

30 Alternatively, the compositions may be formulated as functional foods or beverages, in which situation the carriers and excipients will typically comprise edible food or beverage products. Such products may be processed foods for consumption hot, such as ready meals, but will more preferably be cold foods including spreads (e.g margarine or low-fat spreads), soft drinks,

breakfast cereals, breakfast bars, breads, biscuits, icecreams, chilled desserts such as yoghurts, mousses or trifles, milk or milk based drinks.

5 Where the peptide active agents present in the compositions of the invention are formulated as functional foods or beverages, it will be important that the maximum dose which can be accidentally consumed by over-eating such foods is not excessive.

10 In one alternative embodiment of the invention, the compositions of the invention comprise or form a bulk non-lamellar phase structure, rather than particles of non-lamellar phase. This is a particularly suitable 15 embodiment for the generation of "depot" compositions which gradually degrade *in vivo* releasing the peptide active agent either as a result of this degradation or by gradual diffusion (or both). Because the compositions of the invention are highly effective in 20 protecting peptide active agents from degredation *in vivo*, these compositions make highly effective depot compositions and precursors. Generally, depot compositions are administered parenterally, especially 25 by intramuscular or subcutaneous injection and may result in release of the active peptide over a period of days, weeks or even several months.

In a depot embodiment, it is generally preferable that the compositions do not adopt a non-lamellar phase 30 structure at the time of administration. This is because bulk non-lamellar phases (as opposed to particles) are often viscous and difficult or painful to inject. As a result, in this embodiment, the compositions typically contain around 0.5 to 50% by 35 weight of an oxygen-containing organic solvent such as an alcohol, ketone, sulphoxide, ester or ether. The addition of such solvents allow the formation of a low-

viscosity pre-formulation which will subsequently form a non-lamellar composition of the invention upon contact with body fluids. Obviously such pre-formulations are also compositions of the invention. In a preferred embodiment, the compositions of the invention thus further comprise 0.5 to 50%, preferably 2 to 30%, more preferably 5 to 20% by weight of an oxygen containing, biotolerable organic solvent. In this embodiment, it is preferable that the composition is not initially in the form of a non-lamellar phase, but forms such a phase (as described herein) upon contact with a body fluid after administration.

Typical solvents suitable for use in the compositions include at least one solvent selected from alcohols, ketones, esters (including lactones), ethers and sulphoxides. Examples of suitable alcohols include ethanol, isopropanol and glycerol formal. Examples of ketones include acetone and n-methyl pyrrolidinone. Suitable ethers include diethylether, glycofurool and dimethylisobarbide. Suitable esters include ethyl acetate and isopropyl acetate and dimethyl sulphide is as suitable sulphide solvent.

Where the composition is a depot composition or pre-formulation, it is preferable that the neutral amphiphilic component comprises a diacyl lipid, especially a diacyl glycerol, and a phospholipid in proportions 5:95 to 95:5 by weight, preferably 10:90 to 90:10 and more preferably 10:90 to 45:55 diacyl lipid:phospholipid.

Correspondingly, in a further embodiment, the present invention provides a method for administering a cationic peptide to a patient comprising injection of a composition of the invention comprising an organic solvent (as described herein) which subsequently forms a

non-lamellar "depot" composition of the invention in vivo upon contact with a body fluid.

5 The invention will now be further illustrated by reference to the following non-limiting examples.

EXAMPLES:

10 The following abbreviations are used in the Examples below:

	E200	Epikuron 200 (soya lecithin)
	F127	Pluronic™ F127 (BASF)
15	GDO	Glycerol Di-Oleate
	GMO	Glycerol Mono-Oleate
	LA	Linoleic Acid
	LD	Laser Diffraction
	OA	Oleic Acid
20	PC	phosphatidyl choline
	Ret Pal	Retinyl palmitate
	SAXS	Small Angle X-ray Scattering
	sCT	Calcitonin (Salmon)
	TEM	Transmission Electron Microscopy
25	Try Gly	Tri-Glyceride

Example 1 - GMO/OA particles

30 1.1 - Preparation of a dispersion under high pressure

A coarse dispersion of largely cubic particles was formed by mixing Rylo MG 19 GMO (Danisco, 4.70 g) and oleic acid (Apoteket, 0.24 g) and adding the mixture dropwise to poloxamer 147 (BASF, 0.5 g) in deionised water (45.7 g) under vigorous stirring at ambient temperature. The resulting coarse dispersion was

allowed to equilibrate for about 30 minutes before homogenisation in a microfluidizer at high pressure (350 bar) for 10 min (6 cycles) at 40°C.

5 The particle size was measured using laser diffraction (Coulter LS230) before and after homogenisation. The particle morphology and phase behaviour of the homogenisate was analysed using small angle X-ray scattering (SAXS), and by cryo-transmission electron 10 microscopy (cryo-TEM).

The homogenisate was a colloidal dispersion with particle sizes below 1 µm consisting mainly of vesicles with a proportion of cubic-phase-cored particles.

15 Particles prepared by this method were generally exposed to a heat treatment step prior to loading.

1.2 -Preparation of a dispersion under high pressure

20 The procedure of Example 1.1 was repeated, except that the homogenisation step was carried out at low pressure (174 bar) for a shorter period (5 cycles).

25 The low pressure method produced a larger proportion of cubic-phase-cored particles and some vesicles with a bimodal particle size distribution. The homogenisate was optionally exposed to a cycle of heat treatment prior to loading.

30 1.3 - Heat Treatment

An optional cycle of heat treatment is carried out on the non-lamellar dispersion prepared in Examples 1.1 and 1.2 so as to convert a larger proportion of the 35 particles to non-lamellar phase. The particles of the method from Example 1.1 were usually treated with one cycle of heat treatment.

A sample of the dispersion generated in Example 1.1 or 1.2 (10 ml) was autoclaved (121°C, 20 min) and cooled to ambient temperature. When examined by cryo-TEM, a virtually all particles in the dispersion show non-lamellar character. The particle size distribution is also somewhat narrowed in comparison with the dispersion prior to heat treatment and the average particle size increased slightly. The heat-treated particles showed improved stability to storage.

Example 2 - Further non-lamellar dispersions and loading

2.1 - Compositions of non-lamellar dispersions

Non-lamellar dispersions of varying components in water may be prepared by the method of Example 1.1 and optionally treated with the heat treatment method of Example 1.2. The resulting dispersion is analysed for particle size and phase behaviour.

20

No.	Neutral amphiphile(s)	wt % ¹	anionic amphiphile	wt % ¹	Fragmentation agent	wt % ¹	Water % ²	Dominant Phase
1	GMO	94	OA	5	F127	1	91	Cubic
2	GMO	89	OA	20	F127	1	91	Hex ³
3	GMO	49.5	OA	49.5	F127	1	91	Hex ³ + L ₂ ⁴
4	GMO	94	LA	5	F127	1	90	Cubic
5	E200 : GDO	50:32	OA	5	F127	13	90	Hex ³
6	GMO : Ret Pal	73:12	OA	5	F127	10	90	Hex ³
7	GMO : Tri Gly	76:9	OA	5	F127	10	91	Hex ³
8	E200 : GDO	51:33	LA	4	F127	12	91	Hex ³
9	GMO : Ret Pal	73:12	LA	5	F127	12	91	Hex ³

Table 3

¹Weight % of total amphiphile & fragmentation agent

²Weight % water in final dispersion

³Hex = Hexagonal phase

35 ⁴Reversed micellar phase

2.2 - Peptide loading

To each of the dispersions of Example 2.1 is added the cationic peptide desmopressin to a final concentration
5 of 1 mg/ml. The dispersion is allowed to equilibrate for 60 minutes at room temperature.

2.3 Peptide and peptidase inhibitor loading

10 To each of the dispersions of Example 2.1 is added the cationic peptide calcitonin to a concentration of 0.8 mg/ml and one or more peptidase inhibitors selected from aprotinin (Trasylol®), amastatin and/or leupeptin to a total concentration of 0.4 mg/ml. The dispersion
15 is allowed to stand for 3 hours at room temperature..

Example 3 - Peptide degradation by protease trypsin

3.1 Peptide loading and protease assay method

20 Salmon calcitonin (sCT) was dissolved into two vials with 0.9 % saline solution and into one vial with the test non-lamellar dispersion in saline solution, to a concentration of 1 mg sCT/mL. The vials were placed in
25 a water bath at 37°C with stirring.

To one vial with sCT in saline was added an inhibitor, aprotinin (Trasylol®) (10 000 KIE/mL, 125 µL/mL saline).

30 Zero samples were taken out from the two saline vials and from the non-lamellar-dispersion.

The protease Trypsin (500 µg/mL, 30 µL/mL saline) was added to all vials.

35 After fixed time intervals, samples were transferred to HPLC vials and the inhibitor Trasylol® added to the

non-lamellar-dispersion samples and the samples in saline without Trasylol®.

To a 100 µL sample was added 230 µL methanol and between 5 40 and 115 µL chloroform (the volumes being adjusted depending upon the lipid composition and concentration of the non-lamellar-dispersion) so as to form a one phase system. The samples were mixed thoroughly and allowed to stand for five minutes.

10 The one phase system was split into two phases by adding 230 µL chloroform and 700 µL water. The samples were mixed and centrifuged for 5 min at 13 000 rpm. 200 µL of 15 the upper water/methanol phase was transferred to a 300 µL insert vial.

Analysis of the amount of non-degraded peptide was performed using HPLC.

20 3.2 GMO/OA/sCT comparative protease assay

The above loading and test procedure was repeated with non-lamellar dispersion samples containing a varying proportion of oleic acid (OA) as an anionic lipid component, prepared as indicated in Example 1.1 and 1.3 with NaCl added to a final concentration of 0.9% after heat treatment. The controls and non-lamellar-dispersions were treated with s-CT and Trasylol® in the concentrations given above in Example 3.1.

30 The non-lamellar phase was a GMO/OA non-lamellar liquid crystalline phase particle-dispersion ($d \sim 1 \mu\text{m}$) containing 9% lipid to water with GMO/OA ratios of 100/0, 95/5, and 50/50, and 1% polymeric stabiliser 35 Pluronic™ F127 (BASF). Controls were saline solution without Trasylol® and saline solution with Trasylol® as indicated above.

The results of the HPLC analysis are shown in Figure 1, which clearly indicates that increasing the concentration of anionic amphiphile resulted in a decreased degradation rate of the sCT peptide active agent. The 50/50 GMO/OA dispersion may be seen to provide a greater degree of protection than is given even by the peptidase inhibitor Trasylol®.

Example 4 - Animal bioavailability study

10

4.1 - General procedure

15

20

On the first day of the experiment, the rats were prepared by inserting a silicon catheter (OD approx. 1 mm) in the jugular vein under ketalar/xylazin anaesthesia. The catheter was tunnelled under the skin and exteriorised between the scapulae. After surgery the rats were allowed 48 hours of recovery before dosing. The catheter was rinsed with 0.9% NaCl containing 1 mM EDTA, every morning during the recovery period.

25

In the morning, after approximately 16 hours of fasting (water was accessible), the animals were dosed and blood collected. The animals were allowed free access to water after dosing, but had no access to food. After the last sampling, all animals were sacrificed.

30

Non-lamellar particular dispersions were prepared by the high pressure method of Example 1.1 followed by heat treatment as set out in Example 1.3 and calcitonin loading.

4.2 - Dosing

35

The rats were dosed intravenously through the venous catheter or by gavage by a plastic ball-tipped gavage tube. Intravenously dosed rats were given 1 mg sCT per

kg bwt in 0.5 mL/kg of a sterile acetate buffer and
gavaged rats were given dispersions in water of
SCT-containing non-lamellar dispersions to a dose of 0.5
mg SCT per kg bwt. Total non-lamellar dispersions and
5 SCT concentrations were varied and the total oral dose
volume ranged from 2.5 to 25 mg/kg bwt. Oral dosing was
performed under light isoflurane anesthesia.

4.3 - Sampling

10 Blood samples (0.5 mL) were collected pre-dose (one day
before dosing), 10 minutes, 30 minutes, 1 hr, 3 hrs, 6
hrs and 24 hrs after dosing in EDTA-treated test tubes
also containing 500 KIE aprotinin (Trasylol®) per mL
15 sample. All blood samples were gently mixed and held on
ice (maximally 10 minutes) before they were centrifuged
at 2,000 g for 10 minutes at +4 °C. Plasma were then
immediately transferred to new test tubes and put on dry
ice. Samples were stored at -80°C until analysis.

20

4.4 - Analysis

The content of SCT in all plasma samples was measured by
a commercially available enzyme-linked immunoassay kit.

25

Plasma SCT concentration data were utilized to calculate
area-under-the-curve from 0 to 6 hours (AUC) by the
trapezoidal method.

30

Dose-corrected absolute bioavailability of SCT in the
oral non-lamellar formulations were calculated as:

$$\text{Availability (F)} = (\text{AUC}_{\text{oral}} \times \text{Dose}_{\text{IV}}) / (\text{AUC}_{\text{IV}} \times \text{Dose}_{\text{oral}}) \times 100$$

35

4.5 - Results

Rats were dosed with an intravenous SCT solution, and

orally with a GMO/OA (95%/5%) or GMO (100%) sCT non-lamellar dispersions, according to the above described method. All dispersions were predominantly cubic liquid-crystal dispersed phases. Plasma sCT contents were analysed and sCT plasma concentrations were plotted over time (Figure 2). Absolute bioavailability (F) sCT administered orally in the GMO/OA formulation was around 1%, while sCT delivered in the pure GMO formulation resulted in bioavailability of approx. 0.5%. Hence, the GMO/OA (95%/5%) has an enhancing effect of around doubling the oral bioavailability of sCT compared to the non-lamellar GMO (100%) formulation.

15 **Example 5**

Depot composition and in vitro phase study

Injectable formulations containing phosphatidyl choline ("PC" - epicure 200) and glycerol dioleate (GDO) with and without the anionic lipid Oleic Acid (OA) and with EtOH as solvent were prepared to illustrate that liquid crystalline "depot" compositions can be accessed.

Appropriate amounts of PC and EtOH were weighed in glass vials and the mixture was placed on a shaker until the PC completely dissolved to form a clear liquid solution. GDO and optionally OA was then added to form an injectable homogenous solution.

The formulations were manufactured with compositions according to Table 4. A cationic peptide active substance, salmon calcitonin (sCT), was added to each formulation to a concentration of 500 µg sCT/g formulation. The formulations were designed as homogenous suspensions for parenteral administration (mixing required shortly prior to use since the drug is not completely dissolved in the PC/GDO/EtOH system). Formulation G contained OA.

The phase study in this example is performed in excess of rat serum at 37°C in order to simulate an *in vivo* situation. Table 4 shows the same phases formed

5 TABLE 4

Formulation	PC (wt%)	GDO (wt%)	OA (wt%)	EtOH (wt%)	Phase in rat serum
F	36	54	-	10	I _{II}
G	34	51	5	10	I _{II}

10

I_{II} = reversed cubic liquid crystalline phase

OA = Oleic Acid

Example 6

15 In vivo release study from depot formulations

Formulations F and G in Example 5 were used in an *in vivo* drug release study in rat. The formulations were administrated subcutaneously between the scapulae by 20 using a syringe and the dose of sCT was 500 µg/ kg body weight. The release profile was monitored for a period of 13 days. The sCT concentration in the rat plasma samples was analysed with a commercial kit from DSLabs. The drug was enzymatically amplified with sandwich-type 25 immunoassay using biotin-streptavidin as detection system.

Figure 3 shows the results. A pure triglyceride vehicle based on sesame oil was selected as a lipid reference 30 system.

The formulation containing OA displays a slower release of sCT and an improved bioavailability over the 14 days period over which the plasma concentration was followed. This is consistent with an improved stability of the 35 peptide drug..

CLAIMS:

- 1) A composition comprising at least one cationic peptide active agent, at least one neutral structure forming amphiphile, at least one anionic structure forming amphiphile and optionally at least one solvent wherein said composition comprises a non-lamellar phase structure and/or forms a non-lamellar phase structure on exposure to body fluids.
- 10 2) A composition as claimed in claim 1 or claim 2 wherein said non-lamellar phase is a cubic, hexagonal phase or L₃ phase.
- 15 3) A composition as claimed in claim 1 or claim 2 wherein said cationic peptide has an isoelectric point of above 7.0.
- 4) A composition as claimed in any of claims 1 to 3 wherein said cationic peptide is selected from the group consisting of desmopressin, salmon calcitonin and human calcitonin.
- 20 5) A composition of any of claims 1 to 4 which comprises or forms particles of said non-lamellar phase structure.
- 30 6) A composition as claimed in any of claims 1 to 5 wherein the oral bioavailability is at least 1% when measured as blood plasma concentration of active agent relative to intravenous administration in saline solution.
- 35 7) A method for the formation of a composition as claimed in any of claims 1 to 6 comprising forming particles of non-lamellar phase and/or particles which generate non-lamellar phase on exposure to body

fluids, said particles comprising at least one neutral structure forming amphiphile, at least one anionic structure forming amphiphile or salt thereof and optionally at least one solvent, and subsequently contacting said particles with a solution of cationic peptide active agent.

5
8) A composition as claimed in any of claims 1 to 4 further comprising an oxygen containing
10 biotollerable organic solvent.

9) A composition as claimed in claim 8 in the form of a solution which forms a bulk non-lamellar phase upon contact with a body fluid.

15

1/2

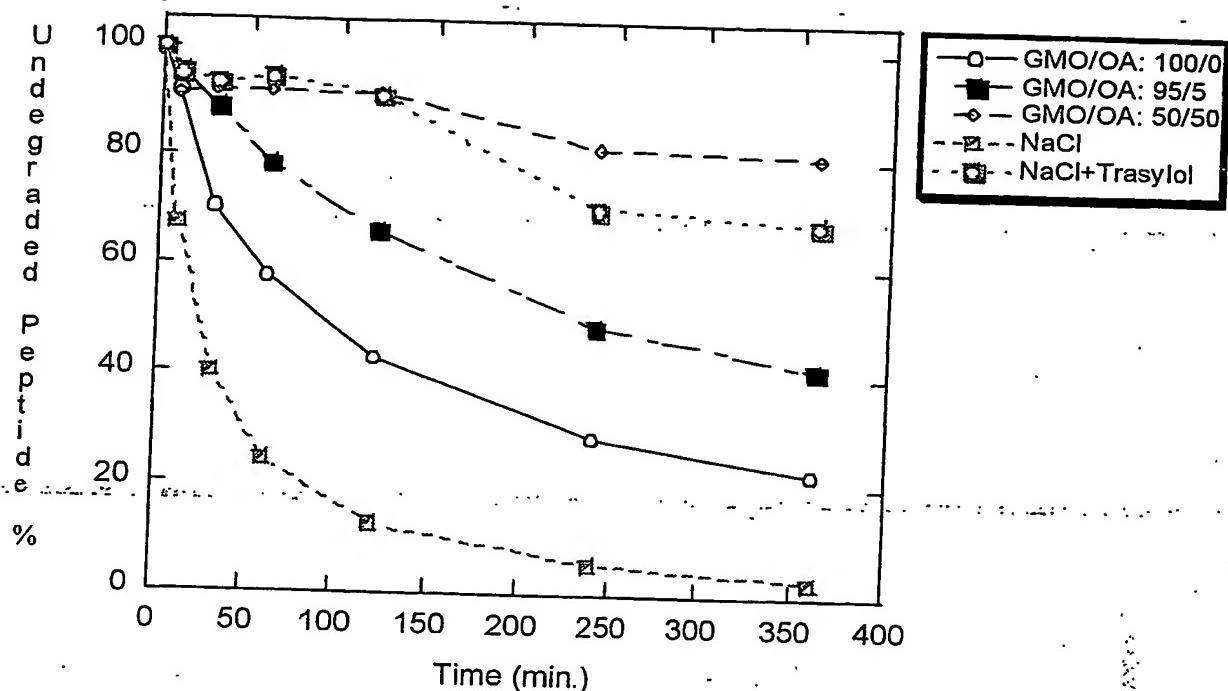


Figure 1 - Proportion of peptide active agent Calcitonin remaining undegraded over time with different formulations.

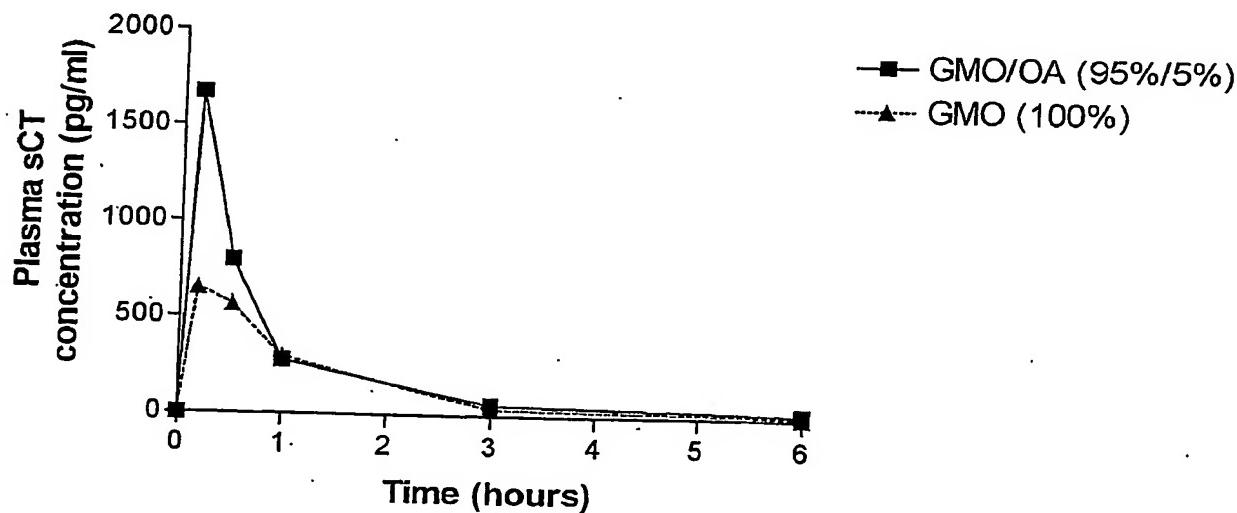


Figure 2. Plasma sCT concentration after oral delivery in a GMO/OA (95%/5%) or GMO (100%) cubic liquid crystalline formulations.



4

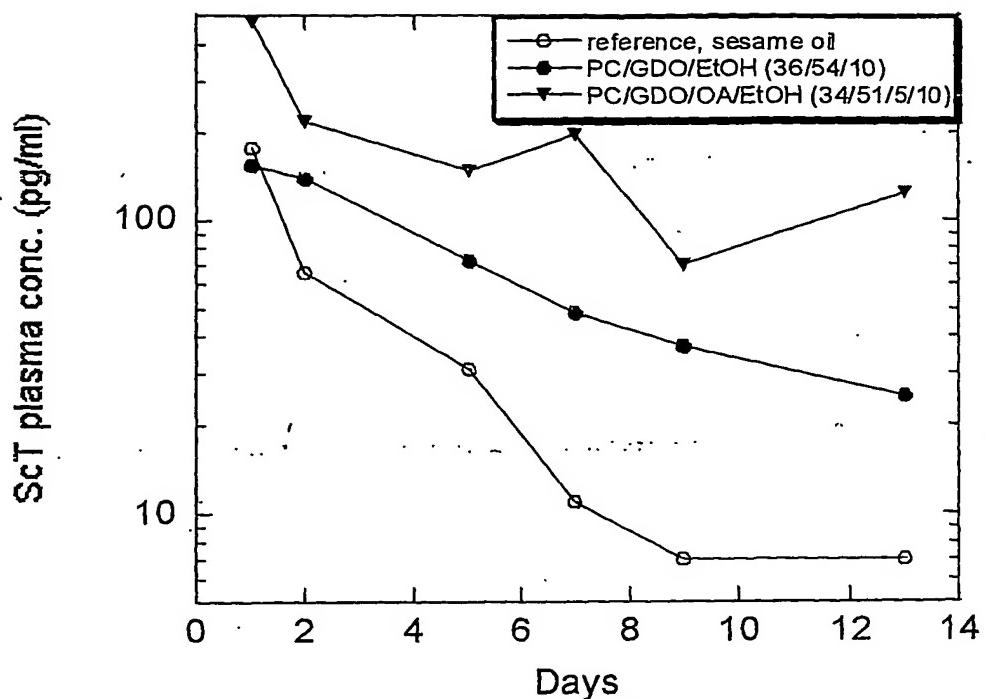


Figure 3. Plasma concentrations in the rat model after subcutaneous administration of depot formulations. A depot based on sesame oil was used as reference.

PCT/GB2004/004696



**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record.**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

BLACK BORDERS

IMAGE CUT OFF AT TOP, BOTTOM OR SIDES

FADED TEXT OR DRAWING

BLURRED OR ILLEGIBLE TEXT OR DRAWING

SKEWED/SLANTED IMAGES

COLOR OR BLACK AND WHITE PHOTOGRAPHS

GRAY SCALE DOCUMENTS

LINES OR MARKS ON ORIGINAL DOCUMENT

REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY

OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.